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Evaluation of different seed dormancy breaking techniques on okra (*Abelmoschus esculentus* L.) seed germination

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Okra (*Abelmoschus esculentus* L.) is one of the horticultural crops commonly grown in Zimbabwe but the productivity of the crop is hampered by poor erratic seed germination due to dormancy. A study was carried out at Bindura University of Science Education to determine the best method and treatment combination of breaking okra seed dormancy. Viability tests and germination tests were conducted first to ascertain that failure of germination was due to dormancy. The study consisted of 3 laboratory experiments arranged as factorial treatment structure laid in a completely randomised design with 3 replications. The 3 experiments consisted of 3 methods of breaking seed dormancy (water soaking, acid scarification and dry heating). Each of the different methods was employed at different exposure duration and at different temperature/concentration levels. Germination was measured for 14 days to determine the total final percentage seed germination. Acid scarified seeds for 3 min at 80% H_2SO_4 concentration level had the best germination percentage of 96.6% followed by dry heating for 5 minutes at 70°C and soaking for 12 h at 30°C which had 92.2 and 91.3% germination respectively. However, H_2SO_4 scarification for 5 min at 60% concentration gave the least germination of 44% followed by soaking for 48 h at 30°C and dry heating for 5 min at 80°C which all resulted in 50% germination. Based on the research findings, 80% H_2SO_4 for 3 min can be used by okra farmers to break dormancy while dry heating for 5 min at 70°C and soaking for 12 h at 30°C are equally good alternatives.

Key words: Dormancy, germination, okra, scarification.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) of the family Malvaceae is a vegetable crop that has achieved tremendous popularity over the last century (Modi et al., 2006). It is grown practically in every country of the world

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and being an important horticultural crop in Zimbabwe, it is grown in the fields, greenhouses and back yard gardens. The crop is tolerant to Zimbabwean mid-season dry spells and its association with the local food in the different regions of the country is creating a great stable demand for it. It can be consumed after cooking as green pods and it is also used after dehydration (dried) and the whole pod is eaten. According to Baskin et al. (2001), aside from being tasty, okra is a very good source of vitamin A and C which are important for bone growth, cell division, and differentiation and are also important in formation of collagen, a protein that gives structures to bones, cartilage, muscles and blood vessels respectively.

Okra plants are mainly propagated by seed. Keller and Kollmann (1999) reported that germination is a critical stage in the life cycle of weeds and crop plants and often controls population dynamics with major practical implications. However, hard seed coats in okra may cause slow and erratic germination and emergence which is exacerbated at sub-optimal conditions (Demir, 2001). Poor and delayed seed germination due to dormancy is one of the major challenges in the propagation of this crop. According to Mohammadi et al. (2012), okra crop exhibits seed hardness that complicates its management, and this seed hardness interferes with seed germination, weed control, harvesting and other management factors. The percentage of seed germination of okra is relatively low, due to occurrence of seed hardness in this plant (Luis-Felipe et al., 2010).

Early germination and plant establishment on the field is essential for timely harvesting and marketing of the vegetable (Denton et al., 2013). Several methods, including heat treatment, chemical (acid) and mechanical scarification can be used to open the seed coat (Mavengahama and Lewu, 2012). Various pre-treatments, such as chemical and physical treatments were tried and reported that only scarification at the radicle end improved the germination of seeds (Ochuodho et al., 2004). Demir (2001) also reported that mechanical scarification is tedious and time-consuming while heat treatments have greater potential for commercial application and have been found to be effective in improving both germination rate and capacity of okra.

A. esculentus growers in Zimbabwe both communal and commercial farmers continuously face challenges from the poor and erratic seed germination of this cultivar, where some of the seed germinate and the other remained in the soil for weeks and sometimes months. This lack of uniformity in germination in most cases forced the farmers to re-sow. Since physical dormancy in okra results in poor germination, crop stand is in turn affected, yield is also affected hence productivity is reduced which leads into losses. The present study was therefore undertaken with the main objective of determining the best method of breaking okra seed dormancy which can be utilised by both smallholder and commercial farmers.

MATERIALS AND METHODS

Study site

The experiment was carried out in 2012 at Bindura University, Astra Campus, in the Biology Laboratory (altitude 1100 m a.s.l.; latitude 17°8'S and 31°19'E), located about 88 km north-east of Harare. The site lies in Natural Region IIa of Zimbabwe's Agro-ecological Zones, characterized by mean annual rainfall of 750 to 1000 mm and mean annual temperature of between 15 and 20°C.

Experimental design and procedure

The study consisted of 3 laboratory experiments, arranged as factorial treatment structure laid out in a completely randomised design (CRD). Each of the treatments of the 3 experiments was replicated thrice.

Seed viability and dormancy tests

Prior to the 3 experiments, a tetrazolium test was conducted to estimate seed viability of all accessions before proceeding with dormancy-breaking treatments. Thirty seeds from the sample were preconditioned by soaking in distilled water at 28°C for 3 h and dissected longitudinally and medially through the embryo. The seeds were then soaked in 1% tetrazolium solution for 1 h at 40°C in the dark, and then washed several times with distilled water to remove excess solution. Seeds were considered viable when the embryo was completely stained, or when the only extremities of the scutellum and/or the tip of the radicle remained unstained. Seed viability was determined at 90%. Seed dormancy was determined by germination of intact seeds in 9 cm Petri dishes lined with filter paper and with distilled water, in an incubator at a temperature of 30°C. The germination of seeds was monitored daily over a period of 14 days. Germination was scored as the emergence of the radicle that reached 2 mm. The seeds were considered to be strongly dormant since 18 seeds out of 30 seeds germinated so the determined germination rate was 60%.

The okra seeds used in the experiments were obtained from local farmers which were harvested between November 2011 and February 2012. The seeds were field dried and kept at room temperature. The acquired seeds were kept in the laboratory for 7 days in the open for them to equilibrate at room temperature of about 28°C and 60 to 80% relative humidity. Seeds were assessed for quality by taking note of the seed size, colour, physical damage and physiological maturity. Seeds which were not damaged physically, of the same size and looked healthy were selected and from this group a working sample was taken.

Experiment 1 (Water soaking)

The first experiment evaluated the effect of soaking on breaking okra seed dormancy. It had 2 factors namely water temperature and soaking duration. Temperature factor had 4 levels (15, 20, 25 and 30°C) whilst soaking duration factor had 3 levels (12, 24 and 48 h) hence a 3×4 factorial arrangement. For each treatment combination, 90 seeds were soaked in 100 ml beakers with water at a constant appropriate temperature for different durations; these were placed in the water bath at which the temperatures were controlled. The dishes were being kept in darkness. After the required time seeds were removed from water then surface dried. Seeds were sun dried for 5 h then stored.

Experiment 2 (Acid scarification)

The second experiment evaluated the effect of sulphuric acid

(H_2SO_4) scarification on breaking okra seed dormancy. It had 2 factors namely acid concentration and exposure duration. H_2SO_4 concentration had 3 levels (60, 70 and 80%) while exposure duration had 3 levels as well (3, 5 and 8 min) hence a 3×3 factorial arrangement. For each treatment combination, 90 seeds were placed in beakers and the different concentrations of the acid were added to them. A stop watch was used to measure the time to which these were exposed to the H_2SO_4. After exposure the seeds were removed from the acid just before any acid penetrated the seed coats. When the allocated time was finished, the seeds were removed promptly and washed thoroughly in several changes of water to neutralize completely all remaining acid. After treatment and a thorough washing, the seeds were dried and stored awaiting germination tests.

Experiment 3 (Dry heating)

The third experiment evaluated the effect of dry heating on breaking okra seed dormancy. It had 2 factors namely temperature and exposure duration. Temperatures had 3 levels (60, 70 and 80°C) while exposure duration had 3 levels as well (3, 5 and 8 min) hence a 3×3 factorial arrangement. Dry heat was used, and the temperatures required were more suitable to an oven. For this seed coat treatment the seeds were placed in shallow containers in a preheated incubator oven. After the treatment, the seeds were cooled immediately and then stored. In this experiment the seeds were exposed to varied temperatures at different times in which a stop watch was used to measure the time.

Germination tests

Seeds from the three different methods of breaking dormancy were taken and subjected to germination tests to determine whether the treatments would improve germination percentage. The intact seeds were germinated by putting them in 9 cm Petri dishes lined with filter papers. Distilled water was added to moisten the filter paper and placed in an incubator at a temperature of 30°C. The germination of seeds was monitored daily over a period of 14 days. Germination was scored as the emergence of the radicle that reached 2 mm. Each germinated seed was removed from the dishes in order to avoid mix-up in counting.

Data collection and analysis

Preceding recording of germinated seeds was done on daily bases and the data of the replicates was recorded on different columns for analysis. The final numbers of germinated seeds were collected as germination percentages. The data was subjected to an analysis of variance using GenStat statistical package at 5% significance level.

RESULTS

There were significant differences ($p<0.05$) in the percentage of germinated okra seeds among the different treatment combinations in all the 3 experiments. Significant variations ($p=0.048$) were observed on the first experiment; soaking okra seeds in water for 12 h at 30°C resulted in highest seed germination of 91.3% followed by soaking the seeds for 12 h at 25°C which had 75.22%. On the other hand, soaking the seeds in water for 48 h at 30°C gave the least seed germination of 50% (Table 1).

On the second experiment, significant variations ($p=0.037$) in seed germination were noted among the different treatment combinations when chemical scarification using H_2SO_4 was employed to break okra seed dormancy at different concentrations for different exposure durations. Highest percentage okra seed germination (96.6%) was observed when 80% H_2SO_4 concentration was used for 3 minutes followed by exposing the seeds for 5 minutes in 80% H_2SO_4 which gave 75.6% seed germination. Least seed germination was recorded when 60% H_2SO_4 was exposed to the seeds for 5 min (Table 1).

Significant differences ($p=0.028$) in percentage of germinated okra seeds was also noted when dry heating was used to break okra seed dormancy at different temperatures and different exposure times. Highest seed germination (92.2%) was observed when dry heating was used at 70°C for 5 min while least germination (44.4%) was recorded when the seeds were dry heated for 5 min at 80°C (Table 1).

DISCUSSION

In the water soaking method (Experiment 1), soaking okra seeds in warm water at 30°C for 12 h was the most effective treatment combination for enhancing germination of *A. esculentus*, with germination of 91.33%, thus 31.33% better than the baseline germination of 60%. Higher soaking time at the same duration resulted in a dramatic reduction in germination. This is consistent with Naidu et al. (1999) who reported that a temperature of 30°C was most suitable for germination since most of the enzymes in okra are activated and are at optimum under temperature conditions of between 28 and 30°C. These findings are similar to Ekpong (2009) where soaking *Cleome* seeds for 12 h gave best germination as compared to 24, 36 and 48 h. The soaking for 12 h treatment seems to have promoted the leaching of germination inhibitors on the tastae of okra seeds (Xia and Kermode, 2002). However, soaking the okra seed for longer period (48 h) tended to decrease germination. This may be attributed to water trapped in tissue between the embryo and seed coat creating an oxygen barrier (Reisman-Berman et al., 1989). Moreover, Norton (1986) concluded that anoxia caused by prolonged soaking of seeds may result in irreversible injury due to accumulation of toxic metabolites hence poor germination.

In the H_2SO_4 soaking method (Experiment 2), soaking the seeds in 80% H_2SO_4 for 3 min resulted in highest percentage germination. These findings are similar to those by Pahla et al. (2014) who observed that H_2SO_4 promoted highest germination in *Acacia angustissima*. Germination increased with the duration of exposure to H_2SO_4. As also the case with hot water treatments, it seems the length of time that seeds need to be soaked in H_2SO_4 depends on the hardness of the seed (Velempini

Table 1. Effect of different methods of breaking seed dormancy on okra seed germination.

Experiment 1		Experiment 2		Experiment 3	
H_2O soaking	Germination %	H_2SO_4 scarification	Germination %	Dry heating	Germination %
12 h*15°C	62.22^{cde}	3 min*60%	53.30^{ab}	3 min*60ºC	55.60^{b}
12 h*20°C	72.78^{f}	3 min*70%	56.70^{abc}	3 min*70ºC	55.60^{b}
12 h*25°C	75.22^{f}	3 min*80%	96.60^{f}	3 min*80ºC	70.00^{c}
12 h*30°C	91.33^{g}	5 min*60%	50.00^{a}	5 min*60ºC	50.00^{a}
24 h*15°C	68.89^{ef}	5 min*70%	61.10^{cd}	5 min*70ºC	92.20^{d}
24 h*20°C	70.00^{f}	5 min*80%	75.60^{e}	5 min*80ºC	44.40^{a}
24 h*25°C	67.78^{def}	8 min*60%	57.80^{bcd}	8 min*60ºC	50.00^{a}
24 h*30°C	58.89^{bc}	8 min*70%	64.80^{d}	8 min*70ºC	51.10^{a}
48 h*15°C	57.78^{abc}	8 min*80%	64.80^{d}	8 min*80ºC	56.60^{b}
48 h*20°C	60.00^{bcd}	---	---	---	---
48 h*25°C	52.56^{ab}	---	---	---	---
48 h*30°C	50.00^{a}	---	---	---	---
SED	2.569	***SED***	8.14	***SED***	5.13
p-value	0.048	***p-value***	0.037	***p-value***	0.028
LSD	7.928	***LSD***	7.26	***LSD***	10.87
CV%	14.3	***CV%***	11.2	***CV%***	4.2

Means with the same superscript in the same column are not different. Means with different superscripts in the same column are different.

et al., 2003). The effectiveness of H_2SO_4 concentration of 80% could be attributed to successful removal of several lignified layers in the testae, which are packed tightly together and contain water repelling compounds (Baskin, 2003). These layers act as a mechanical (physical) barrier to water absorption and gaseous exchange (Colling, 2009). This improved the germination capacity of the seeds and the time of 3 min, probably, made sure that no other seed structure was damaged by over exposure. Scarification using acid may also enhance germination capacity by increasing the leaching of growth inhibitors from the seed. Baskin (1998) noted that the whole idea behind treating the seeds is to either completely remove the germination impeding seed coat or to reduce its thickness so that the seed could emerge. Removal or reduction in thickness of the seed coat allows the seed to take up water and respiratory gases thus the germination process can be initiated. It is highly probable that treating seeds with H_2SO_4 reduced the thickness of the seed coat compared to the other scarification techniques. Exposing the seeds to concentrated levels of H_2SO_4 can also have a negative effect as it can end up damaging the seed, when the acid can penetrate into the seed via its exposed micropyle (Ells, 1963). This could possibly explain why seeds that were exposed to 80% H_2SO_4 for a longer period of 8 min recorded a lower emergence rate as compared to those exposed for 3 min in the same concentration.

In the dry heating treatment (experiment 3), dry heating for 5 min at 70°C was the most successful since it had mean germination of 92% and significantly different from other means. This shows an increase in the germination from the baseline germination rate of 60% that was found in the control experiment of the research. Dry heat is a form of thermal scarification that causes the rupturing of the seed coat (Serrato-Valenti et al., 1999; Budy et al., 1986). This could have caused the seeds to imbibe water and hence higher germination percentage. It is likely that heat treatment induced disruption of the palisade cells in the chalazal region and that water was absorbed through these cracks, as recorded for some other hard-seeded species (Egley, 1987). These results are similar to the findings of Demir (2001) who observed that heat treatment improved okra seed germination. Exposure for 3 minutes reduced okra seed germination in this experiment. It could be that the okra seeds exposed to dry heat took longer to heat up to the target temperatures than they could have if they were placed in hot water, and there was probably some heat loss when the oven door was opened. Higher temperatures of 80°C also, rather than cause the seed coat to crack, dehydrated the seeds too much, which in turn affected enzyme activation leading to the decline in germination. Baskin (2000) found that dry-heat treatment decreased germination of dormant pearl millet seeds. However, dry heat has been successful in breaking dormancy in some other species.

Conclusion

Okra seed is dormant. Acid scarification of okra seed for 3 min in 80% H_2SO_4 is the most effective treatment

combination of breaking dormancy and enhancing germination since it had the highest germination rate of 96.6% of the three treatment combinations selected from each method. Soaking okra seed in water for 12 h at 30°C and dry heating for 5 min at 70°C also promotes high germination rates but germination is delayed to a period of 12 days. Acid scarification and dry heating are effective methods of breaking okra seed dormancy which can be utilised by smallholder farmers.

Conflict of Interest

The authors have not declared any conflict of interest.

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